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Radiation Sensitivity of *Listeria monocytogenes* on Beef as Affected by Temperature

ABSTRACT

Longissimus dorsi from beef was inoculated with *Listeria monocytogenes* and the effect of gamma irradiation on the survival of this pathogen at −60 to +15°C was determined. Radiation D-values were determined for inactivation of *L. monocytogenes* at 5°C intervals from −20 to 5°C. These data were used to develop an equation to predict the response to gamma radiation within that range. An abrupt increase in resistance occurred at −5°C. The radiation D-value was 0.45 kGy at 0°C, 0.77 kGy at −5°C and 1.21 kGy at −20°C. A straight line was obtained when the $\log_{10}$ of the D-values from −5 to −20°C was plotted vs the reciprocal of the absolute temperature. This led us to calculate a value analogous to the Arrhenius activation energy for inactivation of *L. monocytogenes* by gamma radiation.

Key Words: beef, irradiation, food safety, *Listeria*, meat

INTRODUCTION

LISTERIA MONOCYTOGENES has caused periodic outbreaks of foodborne disease with considerable mortality and is of particular concern in ready-to-eat refrigerated foods because it can multiply at refrigeration temperatures (Broome et al., 1990; Palumbo, 1986). This concern caused the Food & Drug Administration and the Food Safety & Inspection Service of the U.S. Department of Agriculture to establish zero tolerances for *L. monocytogenes* in ready-to-eat foods. Most outbreaks of listeriosis were related to products such as cole slaw, milk, and cheese; however, undercooked wieners, sausages, poultry, and shellfish also have been associated with outbreaks of the disease (Cantoni et al., 1989; Centers for Disease Control, 1989; Pearson and Marth, 1990; Schwartz et al., 1988). *L. monocytogenes* have been found on raw meat and poultry and on their products such as paté, turkey franks, fermented sausages, and cooked beef (Bailey et al., 1989; Carosella, 1990; Farber et al., 1989; Genigeorgis et al., 1989; Roberts, 1994; Wenger et al., 1990).

Gamma or electron-beam irradiation treatment of meat was proposed as a method for elimination of *L. monocytogenes* (Beuchat et al., 1993; Grant and Patterson, 1992; Huhtanen et al., 1989; Patterson, 1989; Patterson et al., 1993). With exception of Beuchat et al. (1993) no other workers considered the effects irradiation temperature might have on survival of the organism. Because of possible effects temperature may have introduced (from 2–4°C to 12°C, Huhtanen et al., 1989; Patterson, 1989; Patterson et al., 1993), it was not valid to compare results from these studies. Beuchat et al. (1993) reported on the survival of *L. monocytogenes* on ground beef at irradiation temperatures from −17 to −15°C and from 3 to 5°C but did not find a significant temperature effect. However, significant effects of irradiation temperature were found on the survival of *Campylobacter jejuni* (Clavero et al., 1994), *Clostridium botulinum* spores (El-Bisi et al., 1966), *Escherichia coli* O157:H7 (Clavero et al., 1994; Thayer and Boyd, 1993; Stapleton and Edington, 1953), *Salmonella* (Thayer and Boyd 1991a,b; Thayer et al., 1990), and *Staphylococcus aureus* (Thayer and Boyd, 1992). Because temperature-dependent radiation sensitivity for both gram-positive and negative bacteria has been demonstrated, we initiated a study of the effects of irradiation temperature on survival of *L. monocytogenes*. Our objective was to test the "null hypothesis" that the resistance of *L. monocytogenes* to gamma radiation was not affected by the temperature of the sample during irradiation.

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MATERIALS & METHODS

Cultures

Listeria monocytogenes 15313, 43256, 49594, and 7644 were obtained from the American Type Culture Collection, Rockville, MD. All cultures were maintained and cloned on Tryptic Soy Agar (TSA, Difco, Detroit, MI). Culture identity was confirmed by gram stains and from reactions on GNI or GPI cards of the Vitek AMS Automicrobic System (bio-Mérieux Vitek, Inc., USA, Hazelwood, MO) (Aldridge et al., 1977; Knight et al., 1990), as appropriate. Each member of the pathogen mixture was propagated independently in 100 mL of tryptic soy broth (TSB, Difco, Detroit, MI) at 35°C with agitation at 150 rpm on a rotary shaker for 18 hr at 35°C. Equal amounts from each culture were mixed, and cultures were harvested by centrifugation. A ten-fold inoculum was prepared by resuspending the cells in 1/10 volume of Butterfield's phosphate (0.25M KH_2PO_4 adjusted to pH 7.2 with NaOH).

Substrates and packaging conditions

Beef (steer) was obtained the day after slaughter from Carl Venezia of Conshohocken, PA. The Longissimus dorsi was carefully trimmed of fat, cubed, and frozen in dry ice. The meat was then pulverized while frozen in a Hobart silent cutter to yield a homogeneous material. The meat was subdivided into 100 ± 0.05g amounts, spread thinly, and vacuum sealed in Stomacher 400 polyethylene bags (Tekmar Co., Cincinnati, OH). The bags were then vacuum sealed in high barrier pouches fabricated with 0.025 mm polycaprolactam (nylon 6) as the outside layer, 0.0090 mm aluminum foil as the middle layer, and 0.051 mm polyethylene terephthalate as the inner layer (American National Can Company, Des Moines, IA) to provide better protection during handling and to prevent oxygen transmission to the samples. The meat was frozen at −50°C and sterilized by gamma irradiation to a dose of 42 kGy at −30°C. Prior research (Thayer et al., 1987, 1990; Thayer and Boyd, 1991a,b) demonstrated that such treatments did not significantly alter the wholesomeness and nutritional characteristics or the response of *Salmonella typhimurium* on chicken meat to gamma radiation. Both the sterile and nonsterile meat were stored at −50°C prior to sterilization and use.

Radiation source and irradiation techniques

The self-contained gamma-radiation source of ^{137}Cs had a strength of ~134,000 Ci (4.95 PBq) and a dose rate of 0.108 kGy min^{-1}. The dose rate was established using National Physical Laboratory (Middlesex, U.K.) dosimeters. Variations in absorbed doses given to experimental samples were minimized by placement within a uniform portion of the radiation field. Samples were maintained at the selected temperature ±0.5°C during irradiation by injecting the gas phase from liquid nitrogen into the irradiation chamber. Temperature was monitored continuously during irradiation with a calibrated thermocouple placed directly on the sample.

Inoculation of meat for determination of D_{10} values

Sterile meat was inoculated with enough cells for a final population of ~10^9 stationary-phase cells/g (10 mL per 100g meat) and mixed in a sterile Number 400 polyethylene Stomacher bag for 90 sec using a Stomacher 400 (Tekmar Co., Cincinnati, OH). Aliquots of 5.0 ± 0.05g of

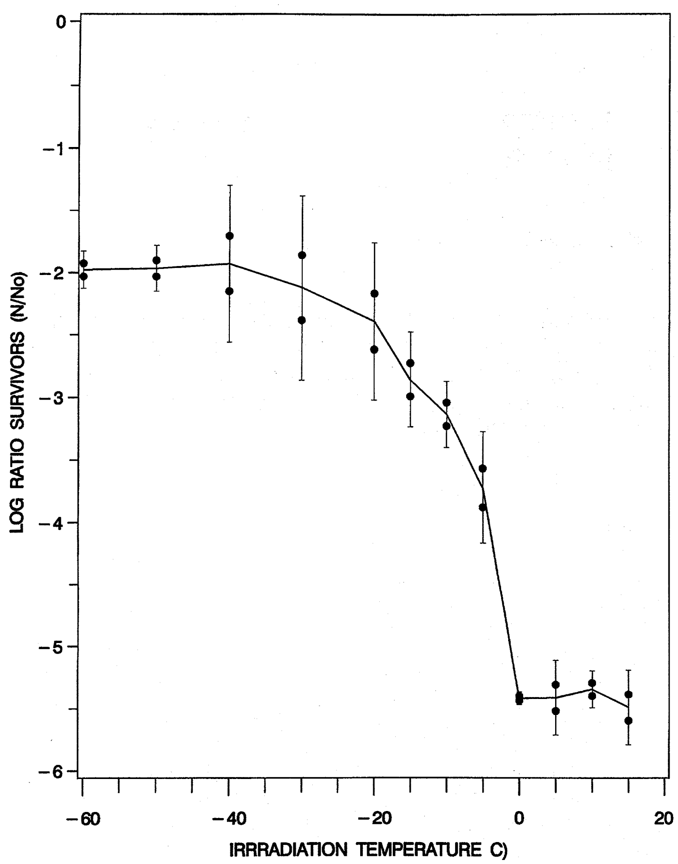

Fig. 1—Survival of *L. monocytogenes* on ground beef following gamma radiation dose of 2.0 kGy as related to temperature during irradiation.

inoculated meat were transferred aseptically to radiation-sterilized oxygen-permeable poultry bags (E-300, Cryovac Division, W.R. Grace & CO., Duncan, SC). The bags complied with U.S. regulations. Inoculated meat was spread uniformly over an area of about 10 × 10 cm within the bags and heat sealed *in vacuo*.

Effect of irradiation temperature on survival

Inoculated meat samples received a dose of 2.0 kGy at irradiation temperatures of 15, 10, 5, 0, −5, −10, −15, −20, −30, −40, −50, and −60°C. Two nonirradiated controls were prepared from the same inoculated meat; one was frozen and one was not. Controls were used to determine the number (N_0) of *L. monocytogenes* colony forming units (CFU) prior to treatment with gamma radiation for samples irradiated while nonfrozen or frozen. The frozen control tested for any effect due to freezing on viability of cells. The study was repeated twice.

Effect of irradiation temperature on D_{10} value

Inoculated meat samples received radiation doses of 0 to 3.0 kGy in increments of 0.60 kGy at 5, 0, −5, −10, −15, and −20°C. All samples for each replicate study were inoculated from the same inoculum. The studies were repeated twice.

Microbiological analysis

Samples were assayed for CFU by standard pour-plate procedures using TSA with serial dilutions in sterile Butterfield's phosphate. Petri plates containing *L. monocytogenes* were incubated for 48 hr at 37°C before counting. CFU were counted on three petri plates having 30–300 colonies with a New Brunswick Scientific Biotran II automated colony counter.

Statistical analysis

Cultural responses were expressed as the logarithm of CFU/g. For each experiment, the average (N) CFU for the three plate counts obtained for each replicate sample was determined and divided by the average of the three zero-dose values (N_o) to give a survivor value (N/N_o). The $\log_{10}$ survivor values ($\log_{10}(N/N_o)$) were then used for subsequent calculations. The D-values (dose in kGy resulting in 90% reduction of viable CFU at specific temperatures) were the reciprocals of the slopes of linear regressions of the log survivor values vs radiation dose determined by least squares analyses. Zero-dose values were excluded from the calculation of regression to avoid shoulder effects as described by Thayer et al. (1990). Regression techniques were used to fit second-order response-surface models (Draper and Smith, 1981). Statistical calculations were performed with the general linear models procedure of the SAS statistical package (Freund et al., 1986; SAS Institute, Inc., 1987). Regressions were tested for differences by analysis of covariance.

RESULTS

Effect of irradiation temperature on survival

Results of an irradiation dose of 2.0 kGy at irradiation temperatures from −60°C to +15°C were compared (Fig. 1). Almost no change occurred in the resistance of *L. monocytogenes* to gamma radiation between 15 and 0°C; however, between 0 and −5°C a very high increase in resistance occurred. Further increases in radiation resistance occurred between −5 and −40°C.

Effect of irradiation temperature on radiation D-value

Analysis of survival data at various doses, for the 2 replicate studies produced the following equation from which survival of *L. monocytogenes* at any temperature between 5 to −20°C and radiation doses of 0 to 3.0 kGy could be predicted:

$$Log_{10}\ survivors\ (N/N_0) = -0.1001 - 0.0650 \times temperature - 1.922 \times kGy - 0.0638 \times temperature \times kGy - 0.0036 \times temperature^2 \quad (1)$$

R^2 for this equation is 0.9470. With it we could make predictions as indicated (Fig. 2). Using Eq. (1) the logarithm of the predicted survival (N/N_o) of *L. monocytogenes* when irradiated to an absorbed dose of 2.4 kGy on beef at temperatures of −20, −15, −10, −5, 0, and 5°C is −1.791, −2.251, −2.892, −3.712, −4.713, and −5.894, respectively.

The D-value for *L. monocytogenes* did not change between irradiation temperatures of 5 and 0°C but increased almost three-fold between 0 and −20°C (Table 1). When the $\log_{10}$ of radiation D-values for both replicate studies at each temperature were plotted vs the reciprocal of absolute temperature (K°) from −5 to −20°C they fell in a straight line (Fig. 3), from which the following regression equation with $R^2 = 0.953$ could be calculated where T = K°:

$$\log_{10} D\text{-}value = -3.539 + 917.3 \times (T^{-1}) \quad (2)$$

Eq. (2) could be used to calculate D-values at intermediate temperatures; but, more importantly, the slope could be converted to a value analogous to the Arrhenius activation energy (E_a):

$$k = se^{\frac{-E_a}{RT}} \quad (3)$$

Where s is a constant, E_a is the Arrhenius activation energy, R is the gas constant, and T is the absolute temperature (K°). If D-value is substituted for the rate constant and Eq. (3) is expressed in logarithmic form,

$$\log D\text{-}value = \frac{E_a}{2.303R} \times \frac{1}{T} + \log s \quad (4)$$

Differentiation of Eq. (4) with respect to temperature and integration yields,

$$\log \frac{D\text{-}value_2}{D\text{-}value_1} = \frac{E_a}{2.303R} \times \frac{T_2 - T_1}{T_1 T_2} \quad (5)$$

Using Eq. (5) the slope of the line (Fig. 3) is 917.3, and E_a has

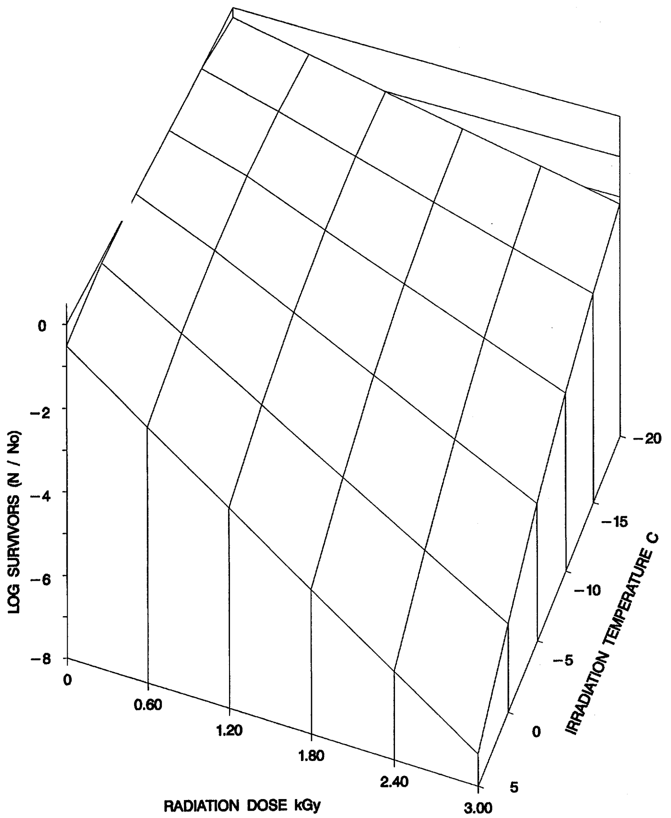

Fig. 2—Predicted survival of *L. monocytogenes* on ground beef following gamma radiation doses of 0 to 3.0 kGy at irradiation temperatures from −20 to +5°C.

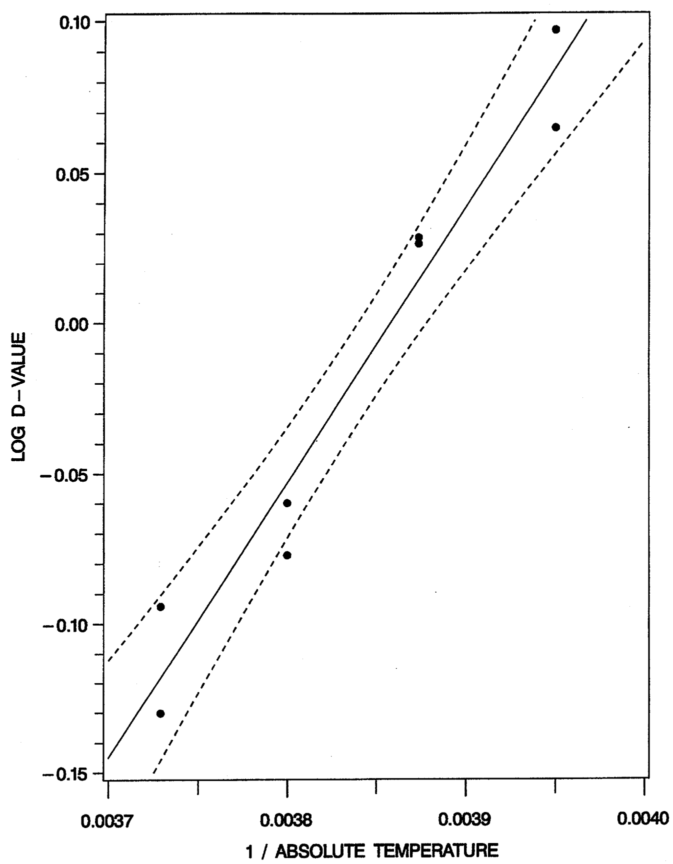

Fig. 3—Log D-values for *L. monocytogenes* on beef treated with gamma radiation as related to 1/T (absolute temperature).

Table 1—Gamma radiation resistance of *L. monocytogenes*[a] at different temperatures

Irradiation temp °C	1/T[b]	D-value ± SE[b] kGy	log D-value
+5	0.00360	0.445 ± 0.012	−0.352
0	0.00366	0.453 ± 0.016	−0.344
−5	0.00373	0.772 ± 0.046	−0.112
−10	0.00380	0.854 ± 0.046	−0.068
−15	0.00387	1.006 ± 0.036	0.028
−20	0.00395	1.208 ± 0.058	0.082

[a] *L. monocytogenes* ATCC 7644, 15313, 43256, and 49594 harvested at 16 hr and mixed with beef *Longissimus dorsi.*
[b] T = absolute temperature; SE = standard error.

the value of −2.303R (slope) = 17,564 joules deg^{-1} $mole^{-1}$. Since the D-values are expressed in kGy per log cell inactivation, and 1 Gy is equivalent to 1 joule/kg, E_a can also be expressed as 17.6 kGy g^{-1} deg^{-1} $mole^{-1}$.

DISCUSSION

RESULTS CLEARLY INDICATE a significant response of *L. monocytogenes* on beef to the temperature of irradiation; $10^{2.9}$ more cells would survive a dose of 2.4 kGy at −20°C than at 0°C. Billen (1987) estimated that ~85% of the potential damage to irradiated *E. coli* was due to radiolysis products of water, primarily OH•. The change in radiation sensitivity at subfreezing temperatures has been attributed to decreased OH•mobility (Taub et al., 1979). Beuchat et al. (1993) did not find a significant difference in radiation resistance of *L. monocytogenes* to gamma radiation on nonfrozen (2–5°C) and frozen (−17 to −14°C) hamburger. We postulate that the discrepancy between our results and those from Beuchat et al. (1993) may be due to recovery and possibly growth of injured *Listeria* in nonfrozen samples during the 12 to 14 hr storage and shipping time before samples (in the Beuchat et al., 1993 study) were analyzed. In our study, samples were analyzed immediately following irradiation, whereas in their study samples had been shipped from Florida to Georgia for analysis.

We obtained a D-value of 0.44 at +5°C, similar to values reported by Huhtanen et al. (1989) at 2–4°C on chicken of 0.46 kGy and Grant and Patterson (1992) at 3–4°C of 0.40 kGy. Equation (1) predicted that the effects of a radiation dose of 2.0 kGy at 3°C would be a reduction in number of surviving CFU by 4.55 log. The mean of the nonirradiated population of Huhtanen et al. (1989) was 8.50, which was reduced to 4.41 by irradiation to 2.0 kGy, a reduction of 4.19 log. The difference between the predicted reduction of viable CFU and the reported value were not great and could result from several factors, such as presence of oxygen during irradiation.

The linear increase in the $\log_{10}$ D-values from −5 to −20°C further supported the hypothesis that inactivation of bacteria even at temperatures below freezing are directly related to reaction temperature as occurs for chemical reactions. This also supports the concept that most cellular inactivation processes are due to interactions with radiolytic products of water rather than direct interaction of gamma radiation with DNA, since those reactions would not be temperature dependent. Our developed Eq. (1) could predict the response of *L. monocytogenes* on beef to gamma irradiation between the temperatures of 5 to −20°C. This equation indicates that products contaminated with *L. monocytogenes* and irradiated to a dose of 2.4 kGy even at −20°C would be much safer than nonirradiated products. However, much greater security could be provided by irradiation at temperatures of 0 to 5°C.

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Ms received 8/29/94; revised 11/22/94; accepted 12/6/94.

We appreciate the technical assistance of K. Snipes and R. Dvorshak.
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